227

HÆMOLYTIC STREPTOCOCCAL INFECTIONS IN THE RABBIT.

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HÆMOLYTIC STREPTOCOCCAL INFECTIONS IN THE RABBIT.

Most workers would probably agree that the rabbit is the most suitable laboratory animal for the investigation of streptococcal problems. In the rabbit it has been found that the pathogenic effects produced by strains of hæmolytic streptococci from scarlatinal and non-scarlatinal conditions do not appear to depend on the origin of the strain in terms of human disease. If small quantities of any of these streptococci are given intradermally, superficial streptococcal abscesses are produced ; if larger quantities are injected or a more virulent culture is used, a spreading condition is produced which may be compared with cellulitis or erysipelas. We have found that the erysipelas-like lesions described by Birkhaug as characteristic of erysipelas strains can be produced by strains of hæmolytic streptococci of the most diverse origin. There has been no evidence of specific blocking of these skin reactions with any serum, although a neutralisation effect was obtained with antierysipelas sera which were kindly sent to us by Dr. Birkhaug or which we had ourselves prepared by Birkhaug's methods. The results with these sera were, however, in the end inconclusive, for interference with the development of skin reactions was frequently induced to the same extent by scarlet fever antitoxic and antibacterial sera, diphtheria antitoxin, tetanus antitoxin, and normal horse serum. The effect, therefore, could not be ascribed to a specific antibody.

Fatal Septicæmia due to the Streptococcus of Scarlet Fever Controlled by Streptococcal Antitoxic Serum.

In previous papers it has been shown that many strains of hæmolytic streptococci from cases of scarlet fever are lethal for rabbits if given in suitable doses, and that this effect can be controlled by the intravenous injection, 4 to 6 hours before the culture, of streptococcal antitoxic serum, but not of sera other than streptococcal (Parish and Okell, 1927). Thus, young broth cultures of the scarlet fever streptococcus (S.F.1), given intravenously, kill rabbits with considerable regularity, the majority dying of acute septicæmia within a few days. Of 120 rabbits which received normal horse serum, concentrated diphtheria or concentrated tetanus antitoxin 4 to 6 hours before the injection of culture, only 20 per cent. survived for 48 hours and 6 per cent. for 6 days (Table I.). Unconcentrated scarlet fever antitoxin in doses of 5 c.cm. or less, and concentrated* antitoxins of average

* By " concentrated " serum is meant serum from which as much as possible of the albumin and euglobulin has been removed by the well-known ammonium sulphate method. It usually represents a three- to four-fold concentration of the protective antibody.

potency in doses of 0·25 c.cm. to 1 c.cm., given intravenously, afford considerable protection against the acute phases of the septicæmia, although the majority of rabbits develop a subacute infection towards the end of the first week or later. This is

TABLE I.

Rabbits injected intravenously with 10 c.cm. of 20-hour digest broth cultures of the scarlet fever streptococcus (S.F. 1). Antitoxic serum given intravenously 4 to 6 hours previously.

Serum.	Total rabbits.	Percent. living six days after injection of serum.			
		C.cm.			
		5 or more.	1·0	0·25	0·1
Normal horse	120	6	..	..	..
Concentrated diphtheria					
,, tetanus					
Unconcentrated scarlet fever (early*)—Batch "P"	9	66	0	..	..
Unconcentrated scarlet fever (later*)—Batch "Q"	15	77	33	..	..
Concentrated scarlet fever—					
Batch "R"	15	88	66	33	..
Batch "S"	12	100	100	66	33
Batch "T"	9	..	100	100	33
Unconcentrated pyogenes	26	27	..	..	..
Concentrated ,, ...	6	66	..	..	..
Unconcentrated puerperal.. ..	32	56	..	..	..
Concentrated ,, ...	13	54	..	..	..
Unconcentrated erysipelas.. ..	14	21	..	..	..
Concentrated ,, ...	10	40	..	..	..

* Stage of immunisation.

characterised mainly by arthritic lesions from which hæmolytic streptococci can readily be recovered. Protection has never been obtained against this phase of the infection with any antitoxic or antibacterial serum we have used.

Many antistreptococcal sera, other than scarlatinal, prepared by the immunisation of horses with filtrates of hæmolytic streptococci have some protective properties in doses of 5 c.cm. or more. Experiments have been carried out with 14 unconcentrated and 4 concentrated antitoxins prepared from pyogenes, puerperal fever, and erysipelas strains. The percentages of rabbits which survived for 6 days ranged from 21 per cent. of those injected with unconcentrated erysipelas to 66 per cent. of those injected with concentrated pyogenes antitoxins. The protection given by the various sera correspond roughly to the amount of streptococcal antibody which they might be expected to contain from collateral evidence. The filtrates which were prepared from pyogenes,

puerperal fever, and erysipelas strains, and which were used for the immunisation of horses, were weaker than those from the scarlet fever streptococcus (S.F.1) as judged by skin reactions in human subjects. It should be emphasised here that we have never been able to demonstrate in any type of experiment that

TABLE II.—*Prevention of Deaths due to Toxin by Antitoxin.*

Rabbits injected intravenously with 30 to 40 c.cm. of 20-hour culture filtrates of scarlet fever streptococcus (S.F. 1). pH 7·6: no preservative. Concentrated antitoxic serum given 4 to 6 hours previously (amounts up to 5 c.cm. intravenously, remainder intraperitoneally.)

Serum.—D.T. = diphtheria or tetanus; S.f. = scarlet fever; Py. = pyogenes; P. = puerperal; E. = erysipelas.

Serum.	Total rabbits.	Percent. living six days after inject. of serum. C.cm. 15	10	5	1	Serum.	Total rabbits.	Percent. living six days after inject. of serum. C.cm. 15	10	5	1
D.T.	30	3	..	..	..	P. ..	3	66	..	..	..
S.f...	3	100	..	..	..		3	..	33	..	..
	3	..	66	..	..		6	..	..	50	..
	12	..	..	75	..	E. ..	3	33	..	..	..
	3	..	..	..	33		6	..	33	..	..
Py...	3	..	100	..	..		3	..	..	0	..
	3	..	..	0	..						
	3	..	..	..	33						

the serum of rabbits highly immunised against washed (toxin free) streptococci, and often rich in agglutinins, has any protective properties. This probably explains the slight protective value shown by most of the older types of horse serum, used in the treatment of human streptococcal infections, which was usually prepared in response to injections of the bacillary bodies.

Deaths due to Scarlet Fever Toxin Prevented by Streptococcal Antitoxin.

Streptococcal antitoxic sera protect rabbits from deaths due not only to culture but also to scarlet fever toxin (Table II.). Although rabbits readily succumb to 5 to 10 c.cm. of young digest broth cultures of many strains of scarlet fever streptococcus, much larger doses—20 to 40 c.cm. given intravenously—of culture filtrates (without preservative), containing at least 15,000 human skin test doses per c.cm., are necessary to ensure deaths within 24 hours. Control injections of 60 c.cm. of the tryptic digest broth used for the preparation of toxin produced only temporary ill-effects. The toxin is almost completely inactivated by heating to 95–100 °C. for 3 hours; 5 of 7 rabbits injected with 40 c.cm. of the heated filtrate survived. When scarlet fever or other streptococcal antitoxin is given intravenously 4 to 6 hours before the injections of toxin the majority of rabbits can be protected,

Table III.

Rabbits injected intravenously with serum 4 to 6 hours before intravenous injection of culture.

Source of culture.	Total rabbits.	Percentage of rabbits living three days (in brackets six days).				
		Unprotected.	Protected: Streptococcus antitoxic serum.			
		No serum or normal horse serum or concentrated diphtheria serum.	Concentrated scarlet fever 5 c.cm.	Concentrated puerperal 5 c.cm.	Unconcentrated pyogenes 10 to 15 c.cm.	Unconcentrated erysipelas 10 to 15 c.cm.
Tonsillitis	24	0	77 (44)	..	33 (16)	..
	9	0	100 (33)	..	33 (0)	..
	6	0	66 (0)	..	..	..
Lymphangitis	9	0	100 (33)	..	66 (33)	..
Fatal septicæmia	12	0	33 (33)	..	66 (66)	..
Severe septicæmia	15	0	66 (50)	..	..	..
Endocarditis	12	0	83 (66)	..	..	..
Fatal endocarditis	12	0	33 (0)	33 (0)	0	..
Puerperal fever—						
Uterus	25	10 (0)	83 (66)	16 (16)	0 (conc. 5 c.cm.)	..
,,	25	0	100 (100)	50 (50)	33 (33) (conc. 5c.cm.)	..
Blood	11	0	100 (33)	100 (33)	..	..
,,	21	17 (0)	83 (66)	66 (50)	66 (0) (conc. 10c.cm.)	..
Uterus	11	0	100 (100)	33 (33)	..	..
Blood	23	17 (17)	100 (100)	66 (66)	66 (0) (conc. 10c.cm.)	..
Erysipelas	36	8 (8)	70 (30)	..	..	77 (22)
	12	0	100 (33)	..	0	0
	12	0	100 (100)	66 (0)	..	66 (33)
	6	0	100 (100)	..	..	..
Total	281					

but, owing no doubt to the fact that the test animal is relatively insensitive to the toxin, the results obtained are so irregular that it has not been found possible to use this method as a practical means of titrating antitoxin in the laboratory. From Table II.

TABLE IV.

Rabbits injected with serum intravenously 4 to 6 hours before intravenous injection of hæmolytic streptococci from conditions other than scarlet fever. (Serum dose—5 c.cm. of concentrated or 15 c.cm. of unconcentrated antitoxin.)

——	Total rabbits.	Percent. survived—	
		Three days.	Six days.
Unprotected.			
No serum	14	0	0
Normal horse serum	24	4·2	4·2
Conc. diphtheria antitoxin ..	59	5·1	1·7
Total	97	4·1	2·1
Protected.			
Scarlet fever antitoxin ..	90	81·1	55·5
Erysipelas antitoxin	20	60·0	20·0
Puerperal fever antitoxin ..	36	52·7	36·1
Pyogenes antitoxin	38	42·1	18·4
Total	184	65·2	40·2

it will be seen that, as a group, the concentrated scarlet fever antitoxins afforded better protection than the various puerperal, pyogenes, and erysipelas antitoxins which were available for the experiments. Concentrated diphtheria antitoxin and concentrated tetanus antitoxin had no protective value.

The experiments which have so far been described demonstrate that sera prepared by immunising horses with filtrates of cultures of hæmolytic streptococci from scarlatinal and non-scarlatinal conditions afford some protection against living cultures of scarlet fever streptococci and their toxins.

Protective Action of Streptococcal Antitoxin against Fatal Septicæmia due to Various Hæmolytic Streptococci.

The acute fatal septicæmias produced in rabbits by strains from cases of acute infectious pharyngitis, suppurative lymphangitis, malignant endocarditis, puerperal fever, erysipelas, &c., can be controlled by scarlet fever antitoxin, and by serum made from toxins from other strains of hæmolytic streptococci. In these experiments the infecting dose for the rabbit ranged from 1 to 20 c.cm. of 9 to 20 hours tryptic digest broth cultures of hæmolytic streptococci from various sources. The dose selected, which was almost always from 1 to 5 c.cm., depended on the virulence of the organisms, and probably represented in most experiments from 2 to 5 times the "minimal lethal dose" for the majority of animals. Serum was given

in doses of 5 to 15 c.cm., usually 5 c.cm. in the case of concentrated antitoxins, and 15 c.cm. of unconcentrated antitoxins. It will be seen from Table III. that scarlet fever, and to a lesser extent other streptococcal antitoxic sera, have considerable protective effect against hæmolytic streptococci of diverse origin, the acute septicæmia which follows the intravenous inoculation of the culture being modified, as in the first series of experiments, into a subacute infection of which arthritis is the most prominent feature. Nearly all the strains have been tested for their ability to produce streptococcal toxin as demonstrated by a Dick test on human subjects, and have been proved to be capable of so doing to a greater or lesser degree.

In Table IV. the results of experiments recorded in Table III. are summarised with reference to the relative protection given by scarlet fever and other streptococcal antitoxins. As shown by the percentages of the total rabbits injected which survived for 3 and for 6 days, scarlet fever antitoxin was of greater value than any of the various samples of erysipelas, puerperal fever, and pyogenes antitoxins which were used for this work.

Summary.

1. Hæmolytic streptococci obtained from various sources—scarlet fever, erysipelas, cellulitis, puerperal fever, &c.—produce similar lesions of a pyogenic nature when injected intradermally in rabbits. We have been unable to demonstrate that sera prepared against either toxin or bacterial bodies contain a specific antibody which will prevent the development of the pyogenic skin reactions.

2. Scarlet fever and other streptococcal antitoxins protect rabbits from the acute phases of the septicæmia following the intravenous injection of the scarlet fever streptococcus, and from deaths due to scarlet fever toxin.

3. They have also a significant protective action against the septicæmia due to hæmolytic streptococci from conditions other than scarlet fever.

4. All hæmolytic streptococci appear to be identical in their "toxic" action. There is strong evidence that the protective action of streptococcal antitoxic sera is due to one antitoxin, which has been produced in horses in varying degree in response to immunisation with toxic filtrates of varying potency.

References.—Birkhaug, K. E.: Bull. Johns Hopkins Hosp., 1925, xxxvii., 307. Parish, H. J., and Okell, C. C., THE LANCET, 1927, i., 71; Jour. Path. and Bact., 1927, xxx., 521.

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